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## Natural Polyelectrolyte Hydrogels with Two Types of Cross-Links with Different Energy

For the first time, gels of a polymer cross-linked by two types of ionic cross-links of different energies, namely trivalent chromium (III) ions and divalent iron (II) ions, were prepared. The negatively charged polysaccharide xanthan, a polyelectrolyte of natural origin, was used as a polymer. A study of xanthan gels cross-linked with each cross-linker separately was carried out to identify the optimal concentrations of each cross-linker for the production of a double cross-linked gel. The mechanical properties of hydrogels under oscillatory shear deformations were then studied. It was demonstrated that the simultaneous use of two cross-linkers resulted in a synergistic increase in the elastic modulus (plateau storage modulus) compared to gels cross-linked with each cross-linker separately. For a gel with two types of cross-links, the elastic modulus was 16 Pa. In contrast, for gels cross-linked with either chromium (III) cations or iron (II) cations, the elastic modulus was approximately 0.6 and 0.5 Pa, respectively. The strong effect can be attributed to the different nature of cross-linking between xanthan macromolecules and chromium (III) and iron (II) cations, as well as the different strength of the cross-links formed. The optimal range of pH values was determined in which a synergistic increase in the elastic modulus of gels with two types of cross-links was observed compared to the corresponding gels cross-linked with each cross-linker separately. Consequently, the concurrent utilization of two cross-linking agents that generate cross-links with disparate energy levels represents an efficacious strategy to improve the tensile strength of polymer hydrogels.

**Keywords:** polymer gel, polysaccharide, xanthan, polymer network, polyelectrolyte, cross-linking, viscoelasticity, rheological properties.

### Introduction

In recent years, the creation and study of hydrogels with high mechanical strength has attracted considerable interest [1–11]. An urgent problem is the search for new ways to increase the mechanical properties of hydrogels so that they can withstand high mechanical loads. One such method is the simultaneous use of several different cross-linkers with different properties. A recently proposed approach in the literature involves the creation of gels with two distinct types of cross-linking: strong covalent and weak non-covalent (“physical”) [12–15]. This approach extends the principle underlying the creation of the so-called “double” networks [16] to single polymer networks: when deformed, strong covalent cross-links maintain gel elasticity, while weak ones break, acting as “sacrificial” bonds, and provide energy dissipation in a sufficiently large volume, which prevents the spread of the break and gel destruction.

A number of networks cross-linked by chemical covalent and “physical” cross-links have been proposed to date. Ionic cross-links are often used as “physical” cross-links [17]. However, there are practically no examples of gels cross-linked simultaneously by two types of ionic cross-links with different energies. This approach appears to offer considerable potential, as the type of ions employed can be varied to alter the ionic cross-linking energy over a broad spectrum.

Thus, the purpose of this work is to prepare polymer gels cross-linked simultaneously with two types of ionic cross-linkers of different energies and to study their mechanical properties in comparison with the corresponding gels cross-linked with each cross-linker separately. The polysaccharide xanthan, which has negatively charged carboxylate groups, was selected as a polymer, and chromium  $\text{Cr}^{3+}$  and iron  $\text{Fe}^{2+}$  cations, which have different charge values and, accordingly, different binding energies with the polymer, were used as cross-linkers.

## Experimental

### Materials

The xanthan gum Ziboxan F200 was procured from the company Deosen Biochemical Limited. The chemical structure of this polysaccharide is shown in Figure 1. Its molar mass amounts to 1,000,000 g/mol (and the degree of polymerization is approximately 1,100), as determined by viscometry in a previous study [18]. Previously reported  $^1\text{H}$  NMR data [18] indicates that the degrees of substitution by acetyl and pyruvate groups, expressed in the number of these groups per monomer link, are equal to 0.56 and 0.41, respectively.

Chromium (III) chloride hexahydrate (purity 98 %), iron (II) sulfate heptahydrate (purity 99 %), sodium azide (purity 99.5 %) provided by Sigma Aldrich were used as received. All solutions were prepared with distilled deionized water from the Milli-Q system (Millipore). The pH values of the samples were adjusted with sodium hydroxide (purity 98 %) from Acros and hydrochloric acid (purity 99 %) from Uralkhiminvest.

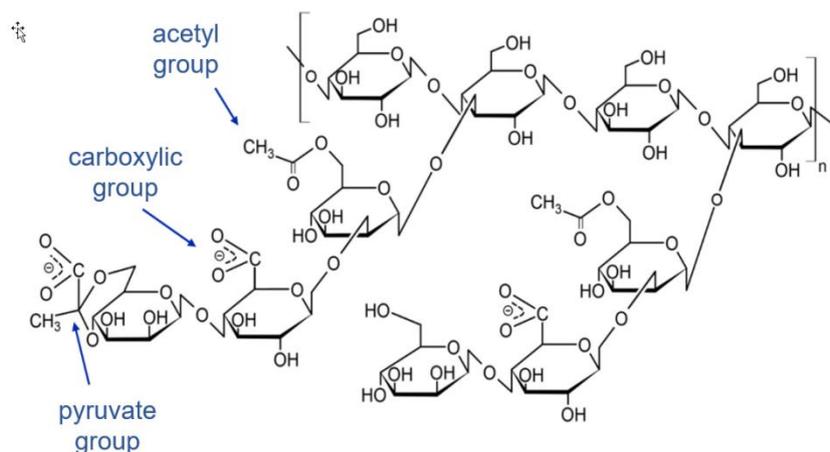


Figure 1. Chemical structure of the polysaccharide xanthan

### Preparation of Samples

Firstly, a 2 wt% aqueous solution of xanthan was prepared by dissolving a calculated amount of the polymer in distilled deionized water in the presence of 3 mM sodium azide, a bacteriostatic agent, at agitation with a magnetic stirrer during 24 h. Secondly, 1 wt% aqueous solutions of cross-linkers (chromium chloride and iron chloride) were prepared by stirring for 5–10 min. Subsequently, the samples were left for 48 hours to allow hydrolysis of  $\text{Cr}^{3+}$  and  $\text{Fe}^{2+}$  ions to proceed, as previously described [19].

Gels with one or two types of cross-links were prepared by simultaneous mixing of aqueous solutions of xanthan, each of the cross-linking agents and acid or alkali (1M KOH or 1M HCl) in required proportions. Then the system was stirred intensively for 2–3 min and left for 7 days for cross-linking. The preliminary experiments indicated that the time needed for cross-linking was the time required for the xanthan solution to gel, i.e. to change from a viscoelastic fluid to a viscoelastic solid, as described in [20].

### Rheometry

The mechanical properties of the hydrogels were studied under shear deformation using an Anton Paar Physica SmartPave 102 rotational rheometer, as described in detail elsewhere [21]. For these experiments, cylindrical gel samples (8 mm in height, 25 mm in diameter) were prepared. The measurements were carried out using a plane-plane measuring cell with a diameter of 25 mm at a temperature of  $20.00 \pm 0.05$  °C. A hood with Peltier elements controlling the temperature was used to prevent solvent evaporation during the experiments.

The measurements were carried out in the harmonic oscillation mode, wherein the frequency dependencies of the storage modulus ( $G'$ ) and loss modulus ( $G''$ ) were recorded at varying frequency  $\omega$  from 0.04 to  $50 \text{ s}^{-1}$ . All experiments were performed in the linear viscoelastic regime at strain amplitudes ( $\gamma$ ) between 1 and 5 %, during which the storage and loss moduli are independent of the amplitude.

### Results and Discussion

In the present work, we prepared and studied hydrogels of xanthan polysaccharide cross-linked simultaneously by two types of ions, namely chromium ( $\text{Cr}^{3+}$ ) and iron ( $\text{Fe}^{2+}$ ). Xanthan monomer links contain negatively charged pyruvate and carboxylate groups, and cross-linking by metal ions occurs mainly by pyruvate groups, although less accessible carboxylate groups can also participate in the cross-linking [22].

Firstly, to determine the optimal conditions for the formation of gels with two types of cross-links, we studied the systems cross-linked by each cross-linker separately with varying concentrations of the cross-linkers. Figure 2 illustrates the dependences of the storage modulus at high frequencies (which, in the case of gels, is analogous to the elastic modulus) on the concentration of each of the cross-linkers. It can be observed that the curve exhibits a similar characteristic appearance for both cross-linkers. At low cross-linker concentrations, the  $G'$  values are low (approximately 0.1 Pa), and no gel formation occurs. Subsequently, when the cross-linker concentration is increased, the storage modulus begins to increase, which corresponds to the onset of gel formation.  $G'$  reaches a plateau at high cross-linker concentrations. This is explained by the formation of cross-links by all available functional groups, which results in the inability to form new cross-links with further increases in the cross-linker concentration.

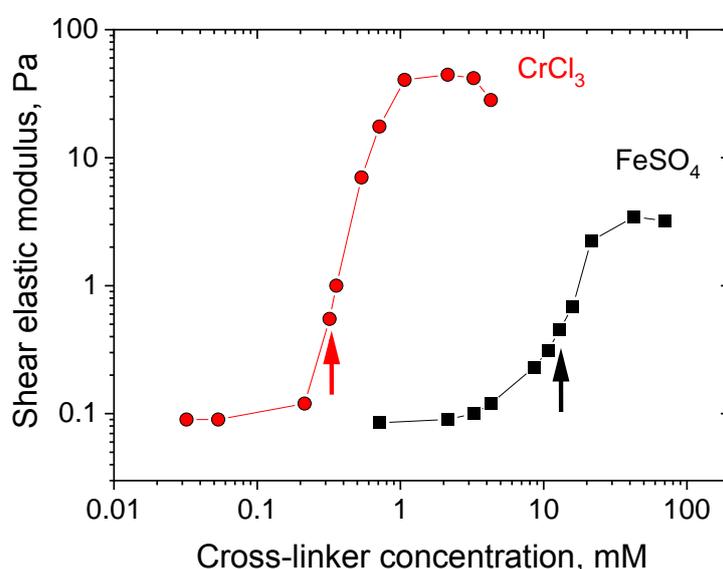


Figure 2. Dependences of the storage modulus  $G'$  at high frequencies (modulus of elasticity) on the cross-linker concentration ( $\text{CrCl}_3$  — circles,  $\text{FeSO}_4$  — squares) for aqueous solutions containing 0.1 wt. % xanthan. Temperature is 20 °C.

It is notable that the curve for  $\text{Fe}^{2+}$  ions is shifted with respect to the curve for  $\text{Cr}^{3+}$  ions in the region of significantly elevated cross-linker concentrations. This phenomenon appears to be associated with a diminished cross-linking efficacy of xanthan by divalent ions relative to trivalent ions [23]. Thus, different concentrations of each of the ions are required to obtain gels with two types of cross-linking. The concentrations indicated by the arrows in Figure 1 (0.32 mM  $\text{CrCl}_3$  and 13 mM  $\text{FeSO}_4$ ), which are close to the onset of the gel formation, were used.

There were obtained transparent, single-phase, and homogeneous xanthan hydrogels cross-linked by both types of cross-linkers simultaneously as well as by each cross-linker separately. Figure 2 shows frequency dependences of the storage modulus and loss modulus for these hydrogels. It can be observed that the addition of a single cross-linker, either  $\text{CrCl}_3$  or  $\text{FeSO}_4$ , results in the formation of gels. This is evidenced by the fact that the  $G'$  values are consistently greater than the  $G''$  values across the entire frequency range. However, the gels are relatively weak, with a modulus of elasticity (storage modulus of accumulation at the plateau  $G_0$ ) of less than 1 Pa. When two cross-linkers are used simultaneously, a gel is also formed, and a synergistic increase in the elastic modulus is observed: for a gel with two types of cross-links  $G_0$  is 16 Pa, while for  $\text{Cr}^{3+}$  and  $\text{Fe}^{2+}$  cross-linkers it is only about 0.6 and 0.5 Pa, respectively. Using the theory for rubber-like elasticity of cross-linked random coil polymers [24] and taking into account the dangling end [24] one can

estimate the average molar mass between network junctions,  $M_c$ , from the plateau modulus  $G_0$  using the following equation [20]:

$$M_c = (KcRT / G_0)(1 + KcRT / G_0M_w),$$

where  $K$  is the empirical factor ranging from 3–10,  $c$  is the polymer concentration in g/l,  $R$  is the molar gas constant,  $T$  is the temperature and  $M_w$  is the molar mass of polymer. With an intermediate  $K$  value ( $K=5$ ), this yields an average molar mass between junctions equal to 200,000 g/mol and 300,000 g/mol for the networks cross-linked by  $\text{Cr}^{3+}$  and  $\text{Fe}^{2+}$  ions separately. This is by several orders of magnitude higher than the molar mass of the repeat unit of xanthan. It indicates that only a small fraction of the ions contributes to the cross-linking process, which is consistent with the previously reported results for ionic cross-linking of xanthan [20]. When two types of ions are used simultaneously, the average molar mass between junctions increases significantly ( $M_c \sim 10,000$  g/mol). It is probable that this synergistic behavior can be explained by the different nature of cross-linking by chromium and iron ions, and by the fact that when xanthan is cross-linked by only one ion, it is near the onset of gelation (because  $M_c$  is only a few times less than the polymer molecular weight). At these conditions, one could expect that some polymer chains and many cross-linkers do not participate in the network formation. The use of two ions results in enhanced gelation, incorporation of more polymer chains into the network, and the appearance of more cross-linking points. This leads to an increase in the elastic modulus. Consequently, the simultaneous use of two cross-linkers allows for a synergistic increase in the elastic modulus of gels compared to systems cross-linked with only one cross-linker.

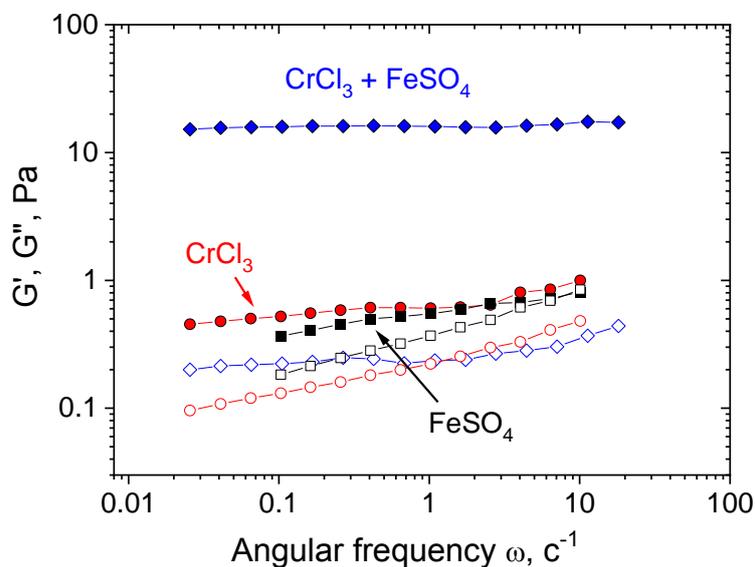


Figure 3. Frequency dependences of the storage modulus ( $G'$ , shaded symbols) and loss modulus ( $G''$ , empty symbols) for aqueous solutions containing 0.1 wt.% xanthan and 0.32 mM  $\text{CrCl}_3$  (circles), 13 mM  $\text{FeSO}_4$  (squares), and both crosslinkers simultaneously (diamonds). Temperature is 20 °C, pH 5.6

One of the determining factors for the formation of ionic cross-links between xanthan molecules is the pH value. Since the optimum conditions for the formation of cross-links by  $\text{Cr}^{3+}$  and  $\text{Fe}^{2+}$  ions appear to be different, the effect of pH on gels cross-linked by both cross-linkers simultaneously and by each of the cross-linkers separately was investigated. Figure 4 shows the pH dependences of the elastic modulus for such gels. It can be observed that the curves for gels cross-linked by two or one cross-linker exhibit a similar characteristic appearance. At low pH ( $< 2.1$ ) the  $G'$  values are rather low, and the gels are not formed. This is attributed to the protonation of the carboxyl groups of xanthan in this pH range, which impedes the interaction of ions with them [25]. At intermediate pH values (from 2.1 to 7.8), gel formation and a sharp increase in the elastic modulus for all three types of gels occur, which is due to the cross-linking of deprotonated carboxyl groups of xanthan by ions. The range in which the gels have the highest modulus of elasticity is narrower for  $\text{Fe}^{2+}$  ions than for  $\text{Cr}^{3+}$  ions. Throughout the pH range from  $\sim 2.1$  to 7.8, a synergistic increase in the elastic modulus is observed as compared to gels with a single cross-linker. Finally, at pH values greater than 7.8, the elastic modulus drops and gels are not formed because both types of  $\text{Cr}^{3+}$  and  $\text{Fe}^{2+}$  ions change into water insoluble hydroxides unable to cross-link the carboxylate groups of xanthan.

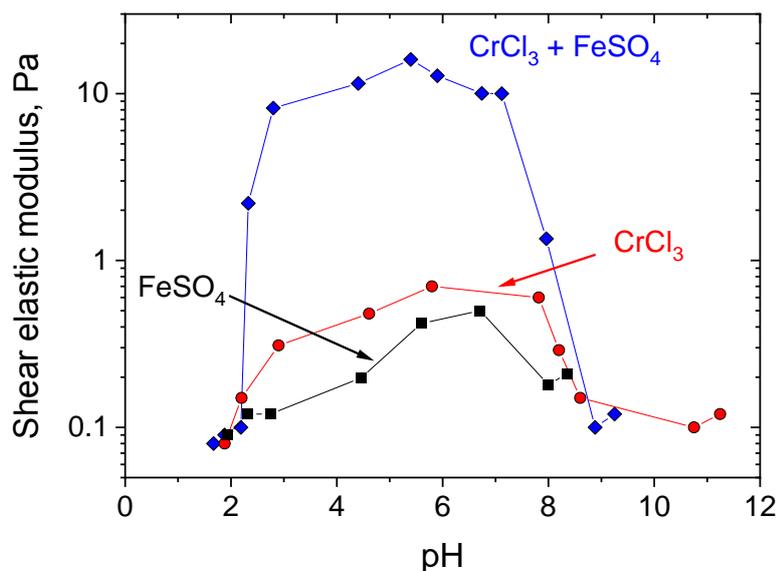


Figure 4. Dependences of the storage modulus  $G'$  at high frequencies (modulus of elasticity) on pH for aqueous solutions containing 0.1 wt.% xanthan and 0.32 mM  $\text{CrCl}_3$  (circles), 13 mM  $\text{FeSO}_4$  (squares), and both cross-linkers simultaneously (diamonds). Temperature is 20 °C

### Conclusions

For the first time, we obtained xanthan hydrogels cross-linked simultaneously by two types of ions,  $\text{Cr}^{3+}$  and  $\text{Fe}^{2+}$ . We also determined the optimum range of cross-linker concentrations and pH, in which a synergistic increase in the elasticity modulus of gels by more than 30 times as compared to gels cross-linked by each of the ions separately was observed.

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### Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. **CRedit**: **Dmitry Aleksandrovich Muravlev** sample preparation, results processing; **Anton Pavlovich Doroganov** sample preparation, rheological measurements; **Olga Evgen'evna**

**Philippova** conceptualization, supervision, writing of the manuscript; **Andrey Vladimirovich Shibaev** data treatment, supervision, writing of the manuscript.

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### Conflicts of Interest:

The authors declare no conflict of interest.

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